CLAIMS

1. A method of detecting a target molecule (20), comprising the steps of:

i) contacting a sample with a locator probe (10,30) comprising a binding moiety (11,31) specific for said target molecule (20) and an amplification nucleic acid sequence (12,32) to produce a target molecule-locator probe complex;

ii) producing an amplification structure bound to any complex produced in the preceding step by performing one or more times the amplification step of treating said sample and locator probe (10,30) with:

a) a single stranded amplification template (40,50) comprising:

i) arranged in a 5' to 3' direction:

a) an extension nucleic acid sequence (41,51);

b) a hybridisation nucleic acid sequence (42) complementary to the amplification nucleic acid sequence (12,32,52) of the previous amplification step or, where there is no previous amplification step, of the preceding step and having substantially the same sequence as said extension nucleic acid sequence (41,51); and

c) an amplification moiety (43,53), being limited in all but the final repeat to a nucleic acid sequence; and

ii) optionally comprising at least one signal moiety being other than a nucleic acid sequence.

b) a polymerising agent capable of extending the 3' terminus of the amplification nucleic acid sequence (12,32,53)of the previous amplification step or, where there is no previous amplification step, of the preceding step by synthesising a complementary strand to said extension nucleic acid sequence (41,51) of said amplification template (40,50);

c) a separating agent capable of removing sufficient of said extension nucleic acid sequence (41,51) of said amplification template (40,50) when hybridised to said complementary strand to allow subsequent hybridisation of said

hybridisation nucleic acid sequence (42,52) of said amplification template (40,50) to said complementary strand; and

d) the reagents and conditions necessary to effect the action of said polymerising agent and separating agent to allow the extension of the 3' terminus of the amplification nucleic acid sequence (12,32,53) of the previous amplification step or, where there is no previous amplification step, of the preceding step by the synthesis of a plurality of sequences complementary to said extension nucleic acid sequence (41,51) of said amplification template (40,50);

iii) detecting any bound amplification template (40,50) from the amplification step or steps; and

iv) correlating the results of detection step (iii) with the presence of said target molecule (20).

2. A method for detecting target molecule according to claim 1, the removal of said extension nucleic acid sequence (41,51) being achieved by the use of a 5' double stranded exonuclease against whose activity the hybridisation nucleic acid sequence (42,52) is protected.

3. A method for detecting a target molecule (20) comprising the steps of:

i) contacting a sample with a locator probe (110) comprising a binding moiety (111) specific for said target molecule (20) and an amplification nucleic acid sequence (112) to produce a target molecule-locator probe complex;

ii) producing an amplification structure bound to any complex produced in the preceding step by performing one or more times the amplification step of treating said sample and locator probe (110) with:

a) a single stranded first amplification template (70,90) comprising:

i) arranged in a 5' to 3' direction:

a) an extension nucleic acid sequence (71,91);

b) a hybridisation nucleic acid sequence (72,92) complementary to the amplification nucleic acid sequence (93,103,112) of the previous amplification step or, where there is no previous amplification step, of the preceding step and having a substantially different sequence to said extension nucleic acid sequence (71,91); and

c) an amplification moiety (73,93), being limited in all but the final repeat to a nucleic acid sequence; and

ii) optionally comprising at least one signal moiety being other than a nucleic acid sequence.

b) a single stranded second amplification template (80,100) comprising:

i) arranged in a 5' to 3' direction'

a) an extension nucleic acid sequence (81,101) comprising said hybridisation nucleic acid sequence (72,92) of said first amplification template (70,90);

b) a hybridisation nucleic acid sequence (82,102) comprising the extension nucleic acid sequence (71,91) of said first amplification template (70,90); and

c) an amplification moiety (83,103), being limited in all but the final amplification step to a nucleic acid sequence; and

ii) optionally comprising at least one signal moiety being other than a nucleic acid sequence;

c) a polymerising agent capable of extending the 3' terminus of the amplification nucleic acid sequence (93,103,112) of the previous amplification step or, where there is no previous amplification step, of the preceding step by synthesising a complementary strand to said extension nucleic acid sequence (71,81,91,101) of said first and second amplification templates (70,80,90,100);

d) a separating agent capable of removing sufficient of said extension nucleic acid sequence (71,81,91,101) of said first and second amplification templates (70,80,90,100) when hybridised to said complementary strand to allow

subsequent hybridisation of said hybridisation nucleic acid sequence (72,82,92,102) of said first and second amplification templates (70,80,90,100) to said complementary strand; and

e) the reagents and conditions necessary to effect the action of said polymerising agent and separating agent to allow the extension of the 3' terminus of the amplification nucleic acid sequence (93,103,112) of the previous amplification step or, where there is no previous amplification step, of the preceding step by the synthesis of a plurality of sequences complementary to said extension nucleic acid sequences (71,81,91,101) of said first and second amplification templates (70,80,90,100);

iii) detecting any bound first and/or second amplification template (70,80,90,100) from the amplification step or steps; and

iv) correlating the results of detection step (iii) with the presence of said target molecule (20).

4. A method of detecting a target molecule (20) according to claim 3, the removal of said extension nucleic acid sequence (71,81,91,101) of said first and second amplification templates (70,80,90,100) being achieved by the use of a 5' double-stranded exonuclease against whose activity said hybridisation nucleic acid sequence (72,92) of said first amplification template (70,90) and said hybridisation nucleic acid sequence (82,102) of said second amplification template (80,100) are protected.

5. A method for detecting a target molecule (20) comprising the steps of:

i) contacting a sample with a locator probe (140) comprising a binding moiety (141) specific for said target molecule (20) and an amplification nucleic acid sequence (142) to produce a target molecule-locator probe complex, said amplification nucleic acid sequence (142) having one or more restriction sites for a restriction endonuclease when hybridised to a complementary strand;

ii) produeing an amplification structure bound to any complex produced in the preceding step by performing one or more times the amplification step of treating said sample and locator probe (140) with:

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a) a single stranded amplification template (120) comprising:

i) arranged in a 5' to 3' direction:

a) an extension nucleic acid sequence (121);

b) a hybridisation nucleic acid sequence (122) complementary to the amplification nucleic acid sequence (123,142) of the previous amplification step or, where there is no previous amplification step, of the preceding step and having substantially the same sequence as said extension nucleic acid sequence (121); and

c) an amplification moiety (123), being limited in all but the final amplification step to a nucleic acid sequence; and

ii) optionally comprising at least one signal moiety being other than a nucleic acid sequence;

b) a polymerising agent capable of extending the 3' terminus of the amplification nucleic acid sequence (123,142) of the previous amplification step or, where there is no previous amplification step, of the preceding step by synthesising a complementary strand to said extension nucleic acid sequence (121) of said amplification template (120);

c) said restriction endonuclease; and

d) the reagents and conditions necessary to:

i) effect the action of said polymerising agent and separating agent to allow the extension of the 3' terminus of the amplification nucleic acid sequence (123,142) of the previous amplification step or, where there is no previous amplification step, of the preceding step by the synthesis of a plurality of sequences complementary to said extension nucleic acid sequence (121) of said amplification template (120); and

ii) effect dissociation of fragments of nucleic acid strands which have been cut by said restriction endonuclease activity from uncut complementary strands whilst not effecting dissociation of uncut nucleic acid strands from uncut complementary strands;

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iii) detecting any bound amplification template (120) from the amplification step or steps; and

iv) correlating the results of detection step (iii) with the presence of said target molecule (20).

6. A method according to claim 5, said amplification nucleic acid sequence (123) and said hybridisation nucleic acid sequence (122) having nucleotide modifications which prevent cleavage by said restriction endonuclease, and said reagents including at least one modified nucleotide which, when incorporated into said complementary strand by said polymerising agent, prevent cleavage of said complementary strand by said restriction endonuclease.

7. A method according to claim 5, said hybridisation nucleic acid sequence having at least one nucleotide modification which prevents cleavage by said restriction endonuclease, said restriction endonuclease having single stranded nicking activity only.

8. A method according to claims 5-7, being performed isothermally.

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9. A method according to claims 5-7, being performed at more than one temperature.

10. A method according to anyone of the preceding claims, the amplification step of step (ii) being performed two or more times.

11. A method for detecting a target molecule (20) comprising the steps of;

i) contacting a sample with a locator probe (150) comprising:

a) a binding moiety (151) specific for said target molecule (20);

b) an amplification nucleic acid sequence (152) to produce a target molecule-locator probe complex; and

c) optionally comprising a signal moiety being other than a nucleic acid sequence;

ii) producing an amplification structure bound to any complex produced in the preceding step by performing one or more times the amplification step of treating said complex with:

a) a single stranded amplification template (160,180) comprising:

i) arranged in a 5' to 3' direction:

a) an extension nucleic acid sequence (162,182); and

b) a hybridisation nucleic acid sequence (161,181) complementary to the amplification nucleic acid sequence (152,173) of the previous amplification step or, where there is no previous amplification step, of the preceding step and having substantially the same sequence as said extension nucleic acid sequence (162,182); and

ii) optionally comprising at least one signal moiety being other than a nucleic acid sequence;

b) a polymerising agent capable of extending the 3' terminus of the amplification nucleic acid sequence (152,173) of the previous amplification step or, where there is no previous amplification step, of the preceding step by synthesising a complementary strand to said extension nucleic acid sequence (162,182) of said amplification template (160,180);

c) a separating agent capable of removing sufficient of said extension nucleic acid sequence (162,182) of said amplification template (160,180) when hybridised to said complementary strand to allow subsequent hybridisation of said hybridisation nucleic acid sequence (161,181) of said amplification template (160,180) to said complementary strand;

d) the reagents and conditions necessary to effect the action of said polymerising agent and separating agent to allow the extension of the 3' terminus of the amplification nucleic acid sequence (152,173) of the previous amplification step or,

where there is no previous amplification step, of the preceding step by the synthesis of a plurality of sequences complementary to said extension nucleic acid sequence (162,182) of said amplification template (160,180); and

iii) optionally repeating one or more times the steps of treating the products of the previous repeat or, where there is no previous repeat, the products of step (ii) with:

a) a separating agent capable of removing the remainder of said hybridisation nucleic acid sequence (161,181) of said amplification template (160,180) of the previous repeat or step (ii) when hybridised to said complementary strand;

b) an additional locator probe comprising:

i) a hybridisation nucleic acid probe (162,182) specific to said complementary strand of the previous repeat or step (ii);

ii) an amplification moiety (173), being limited in all but the final repeat to a nucleic acid sequence; and

to produce a complex; and

c) performing step (ii) as defined above, optionally using an amplification template (160,180) different to that which was previously used;

iv) detecting any bound additional locator probes or amplification template (160,180) from the amplification step or steps; and

v) correlating the results of detection step (iv) with the presence of said target molecule (20).

12. A method for detecting a target molecule (20) comprising the steps of;

i) contacting a sample with a locator probe (150) comprising a binding moiety (151) specific for said target molecule (20) and an amplification nucleic acid sequence (152) to produce a target molecule-locator probe complex;

ii) producing an amplification structure bound to any complex produced in the preceding step by performing one or more times the amplification step of treating said complex with:

a) a single stranded first amplification template (160,180) comprising:

i) arranged in a 5' to 3' direction:

a) an extension nucleic acid sequence (162,182); and

b) a hybridisation nucleic acid sequence (161,181) complementary to the amplification nucleic acid sequence (152,173) of the previous amplification step or, where there is no previous amplification step, of the preceding step and having substantially the same sequence as said extension nucleic acid sequence ; and

ii) optionally comprising at least one signal moiety being other than a nucleic acid sequence;

b) a single stranded second amplification template comprising:

i) arranged in a 5' to 3' direction:

a) an extension nucleic acid sequence comprising said first amplification template hybridisation nucleic acid sequence; and

b) a hybridisation nucleic acid sequence comprising said first amplification template extension nucleic acid sequence; and

ii) optionally comprising at least one signal moiety being other than a nucleic acid sequence;

c) a polymerising agent capable of extending the 3' terminus of the amplification nucleic acid sequence of the previous amplification step or, where there is no previous amplification step, of the preceding step by synthesising a complementary strand to said extension nucleic acid sequence of said amplification template;

d) a separating agent capable of removing sufficient of said extension nucleic aid sequence of said first and second amplification templates when hybridised to said complementary strand to allow subsequent hybridisation of said hybridisation nucleic acid sequence of said first and second amplification templates to said complementary strand;

e) the reagents and conditions necessary to effect the action of said polymerising agent and separating agent to allow the extension of the 3' terminus of

the amplification nucleic acid sequence of the previous amplification step or, where there is no previous amplification step, of the preceding step by the synthesis of a plurality of sequences complementary to said extension nucleic acid sequence of said amplification template;

iii) optionally repeating one or more times the steps of treating the products of the previous repeat or, where there is no previous repeat, the products of step (ii) with:

a) a separating agent capable of removing the remainder of said hybridisation nucleic acid sequences of said first and second amplification templates of the previous repeat or step (ii) when hybridised to said complementary strand;

b) an additional locator probe comprising:

i) a hybridisation nucleic acid probe specific to said complementary strand of the previous repeat or step (ii); and

ii) an amplification moiety, being limited in all but the final repeat to a nucleic acid sequence;

to produce a complex; and

c) performing step (ii) as defined above, optionally using an amplification template different to that which was previously used;;

iv) detecting any bound additional locator probes or amplification template from the amplification step or steps; and

v) correlating the results of detection step (iv) with the presence of said target molecule.

13. A method for detecting a target molecule according to either one of claims 11 or 12, the removal of said amplification template being achieved by the use of a 5' double strand specific exonuclease.

14. A method for detecting a target molecule according to either one of claims 11 or 12, the removal of said amplification template being achieved through the use of elevated temperature.

15. A method for detecting a target molecule according to claim 14, said locator probe being covalently attached to said target molecule prior to the removal of said amplification template.

16. A method for detecting a target molecule according to any one of claims 1-9, prior to said detection step additionally comprising performing a method according to steps (ii) and (iii) of either one of claims 11 or 12.

17. A method for detecting a target molecule according to any one of claims 1-9, said amplification moiety of said amplification template from said final amplification step comprising a nucleic acid sequence, and prior to said detection step additionally comprising performing steps (ii)-(iii) of a method according to either one of claims 11 or 12.

18. A method for detecting a target molecule according to any one of claims 11-15, prior to said detection step additionally comprising performing step (ii) of a method according to any one of claims 1, 3 or 5.

19. A method for detecting a target molecule according to any one of claims 11-15, said amplification moiety of said locator probe or additional locator probe from said final amplification step comprising a nucleic acid sequence, and prior to said detection step additionally comprising performing step (ii) of a method according to any one of claims 1, 3 or 5.

20. A method for detecting a target molecule according to any one of claims 1-9, 18 or 19, the step of detecting any bound amplification template comprising the steps of:

i) treating said sample, locator probe and amplification template or amplification templates with a detection probe which binds specifically to said amplification moiety of the last of said amplification templates; and

ii) detecting any bound detection probe.

21. A method for detecting a target molecule according to any one of claims 11-17, the step of detecting any bound amplification template comprising the steps of:

i) treating said sample, locator probe and amplification template with a detection probe which binds specifically to said amplification moiety of the last of said amplification templates; and

ii) detecting any bound detection probe.

22. A method according to either one of claims 20 or 21, the detection probe having a label which is detected by any one of the group of luminometry, fluorometry, spectrophotometry, and radiometry.

23. A method according to claim 22, the detection probe being labelled with any one of the group of, FAM (carboxyfluorescein), HEX (hexachlorofluorescein), TET (tetrachlorofluorescein), ROX (carboxy-X-rhodamine), TAMRA (carboxytetramethylrhodamine), JOE (carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein), or with biotin.

24. A method according to any one of the preceding claims, the amplification step being performed two or more times, each amplification step being performed using an amplification template having a different extension nucleic acid sequence, hybridisation nucleic acid sequence and amplification moiety to that of the amplification template used in the previous amplification step.

25. A method according to any one of the preceding claims, the target molecule to be detected being a nucleic acid sequence and the binding moiety of said locator probe comprising a nucleic acid sequence complementary to said target molecule nucleic acid sequence.

26. A method according to any one of the preceding claims, being performed using more than one locator probe, each locator probe having the same amplification nucleic acid sequence.

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27. A method according to any one of the preceding claims, comprising two repeats.

28. A method according to any one of the preceding claims, unreacted reagents being removed at the end of step (i), each repeat, or detection step by washing.

29. A method according to claim 28, the unreacted reagents being selected from the group of locator probe, amplification template, and detection probe.

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